In re Application of: GEPSTEIN et al
Serial No.: 10/759734
Filed: January 29, 2004
Office Action Mailing Date: October 19, 2006

Examiner: Singh, Anoop Kumar
Group Art Unit: 1632
Attorney Docket: 27395

In the claims:

1. (Withdrawn) A method of generating cells predominantly displaying at least one characteristic associated with a cardiac phenotype, the method comprising; (a) partially dispersing a confluent cultured population of human stem cells, thereby generating a cell population including cell aggregates; (b) subjecting said cell aggregates to culturing conditions suitable for generating embryoid bodies; and (c) subjecting said embryoid bodies to culturing conditions suitable for inducing cardiac lineage differentiation in at least a portion of the cells of said embryoid bodies thereby generating cells predominantly displaying at least one characteristic associated with the cardiac phenotype.

2. (Withdrawn) The method of claim 1, wherein said culturing conditions suitable for inducing cardiac lineage differentiation include adherence of said embryoid bodies to a surface.

3. (Withdrawn) The method of claim 1, further comprising isolating said cell aggregates from said cell population prior to step (b).

4. (Withdrawn) The method of claim 1, further comprising isolating said embryoid bodies prior to step (c).

5. (Withdrawn) The method of claim 1, wherein said culturing conditions suitable for inducing cardiac lineage differentiation further include culture medium supplemented with serum.

6. (Withdrawn) The method of claim 1, further comprising screening and optionally isolating cells predominantly displaying at least one characteristic associated with a cardiac phenotype, said screening is effected by at least one method

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selected from the group consisting of detection of mechanical contraction, detection of a cardiac specific structure, detection of a cardiac specific protein, detection of a cardiac specific RNA, detection of cardiac specific electrical activity, detection of cardiac specific changes in the intracellular concentration of a physiological ion.

7. (Withdrawn) The method of claim 6, wherein said detection of cardiac specific electrical activity is effected using a microelectrode array.

8. (Withdrawn) The method of claim 7, wherein said multielectrode array comprises electrodes positioned 100 .mu.m or less apart.

9. (Withdrawn) The method of claim 7, wherein said multielectrode array comprises at least 60 electrodes.

10. (Withdrawn) The method of claim 7, wherein said multielectrode array is configured to obtain data characterizing said cardiac specific electrical activity with a frequency greater than a range selected from 1-25 kHz.

11. (Withdrawn) The method of claim 6, further comprising screening and optionally isolating cells substantially displaying proliferation.

12. (Withdrawn) The method of claim 1, wherein said human stem cells are embryonic stem cells

13. (Withdrawn) The method of claim 1, wherein said partially dispersing a confluent cultured population of human stem cells is effected via a non-trypsin based method.

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14. (Withdrawn) The method of claim 1, wherein said partially dispersing a confluent, cultured population of human stem cells is effected via treatment with collagenase.

15. (Withdrawn) The method of claim 1, wherein said culturing in step (b) is effected for a time period selected from the range of 1 to 20 days.

16. (Withdrawn) The method of claim 1, wherein said culturing conditions in step (b) include inhibiting adherence of said cell aggregates to a surface.

17. (Withdrawn) The method of claim 1, wherein said culturing conditions in step (b) include culture medium supplemented with serum.

18. (Withdrawn) The method of claim 1, wherein said culturing in step (c) is effected for at least as long as a time period selected from the range of 1-60 days

19. (Withdrawn) The method of claim 1, wherein said culturing in step (c) is effected in the presence of dimethyl sulfoxide.

20. (Withdrawn) The method of claim 2, wherein said culturing conditions include exposing said embryoid bodies to a surface coated with gelatin.

21. (Withdrawn) The method of claim 1, wherein said at least one characteristic associated with a cardiac phenotype is selected from the group consisting of cardiac specific mechanical contraction, a cardiac specific structure, expression of a cardiac specific RNA, expression of a cardiac specific protein, cardiac specific changes in the intracellular concentration of a physiological ion, cardiac specific electrical activity

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22. (Withdrawn) The method of claim 21, wherein said cardiac specific mechanical contraction is selected from the group consisting of spontaneous mechanical contraction, rhythmic mechanical contraction, synchronous mechanical contraction, and propagative mechanical contraction.

23. (Withdrawn) The method of claim 21, wherein said cardiac specific structure is selected from the group consisting of a sarcomere, a Z-band, a Z body, an intercalated disc, a gap junction, a desmosome, a fibrillar bundle, a fibrillar bundle striation, and a myocytic syncytium.

24. (Withdrawn) The method of claim 21, wherein said cardiac specific RNA encodes a protein selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, .alpha.-actinin, cardiac troponin I, cardiac troponin T, GATA-4, Nkx2.5, MLC-2A, MLC-2V, atrial myosin light chain, ventricular myosin light chain, and connexin-43.

25. (Withdrawn) The method of claim 21, wherein said cardiac specific protein is selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, atrial natriuretic peptide, cardiac troponin I, desmin and connexin-43.

26. (Withdrawn) The method of claim 21, wherein said cardiac specific electrical activity is selected from the group consisting of spontaneous electrical activity, rhythmic electrical activity, synchronized electrical activity, and propagative electrical activity.

27. (Withdrawn) The method of claim 26, wherein said propagative electrical activity is characterized by slow conduction.

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28. (Withdrawn) A method of generating issue predominantly displaying at least one characteristic associated with a cardiac phenotype, the method comprising: (a) partially dispersing a confluent cults population of human stem cells, thereby generating a cell population including cell aggregates; (b) subjecting said cell aggregates to culturing conditions suitable for generating embryoid bodies; and (c) subjecting said embryoid bodies to culturing conditions suitable for inducing cardiac lineage differentiation in at least a portion of the cells of said embryoid bodies thereby generating tissue predominantly displaying at least one characteristic associated with the cardiac phenotype.

29. (Withdrawn) The method of claim 28, wherein said culturing conditions suitable for inducing cardiac lineage differentiation include adherence of said embryoid bodies to a surface.

30. (Withdrawn) The method of claim 28, further comprising isolating said cell aggregates from said cell population prior to step (b).

31. (Withdrawn) The method of claim 28, further comprising isolating said embryoid bodies prior to step (c).

32. (Withdrawn) The method of claim 28, wherein said culturing conditions suitable for inducing cardiac lineage differentiation further include culture medium supplemented with serum.

33. (Withdrawn) The method of claim 28, further comprising screening and optionally isolating tissue predominantly displaying at least one characteristic associated with a cardiac phenotype, said screening is effected by at least one method selected from the group consisting of detection of mechanical contraction, detection of a cardiac specific structure, detection of a cardiac specific protein, detection of a

cardiac specific RNA, detection of cardiac specific electrical activity, and detection of cardiac specific changes in the intracellular concentration of a physiological ion.

34. (Withdrawn) The method of claim 33, wherein said detection of cardiac specific electrical activity is effected using a microelectrode array.

35. (Withdrawn) The method of claim 34, wherein said multielectrode array comprises electrodes positioned 100 .mu.m or less apart.

36. (Withdrawn) The method of claim 34, wherein said multielectrode array comprises at least 60 electrodes

37. (Withdrawn) The method of claim 34, wherein said multielectrode array is configured to obtain data characterizing said cardiac specific electrical activity with a frequency greater than a range selected from 1-25 kHz.

38. (Withdrawn) The method of claim 33, further comprising screening and optionally isolating tissue substantially displaying proliferation

39. (Withdrawn) The method of claim 28, wherein said human stem cells are embryonic stem cells.

40. (Withdrawn) The method of claim 28, wherein said partially dispersing a confluent cultured population of human stem cells is effected via a non-trypsin based method.

41. (Withdrawn) The method of claim 28, wherein said partially dispersing a confluent cultured population of human stem cells is effected via treatment with collagenase.

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42. (Withdrawn) The method of claim 28, wherein said culturing in step (b) is effected for a time period selected from the range of 1 to 20 days.

43. (Withdrawn) The method of claim 28, wherein said culturing conditions in step (b) include inhibiting adherence of said cell aggregates to a surface.

44. (Withdrawn) The method of claim 28, wherein said culturing conditions in step (b) include culture medium supplemented with serum.

45. (Withdrawn) The method of claim 28, wherein said culturing in step (c) is effected for at least as long as a time period selected from the range of 1-60 days

46. (Withdrawn) The method of claim 28, wherein said culturing in step (c) is effected in the presence of dimethyl sulfoxide.

47. (Withdrawn) The method of claim 29, wherein said culturing conditions include exposing said embryoid bodies to a surface coated with gelatin.

48. (Withdrawn) The method of claim 28, wherein said at least one characteristic associated with a cardiac phenotype is selected from the group consisting of cardiac specific mechanical contraction, a cardiac specific structure, expression of a cardiac specific RNA, expression of a cardiac specific protein, cardiac specific changes in the intracellular concentration of a physiological ion, and cardiac specific electrical activity.

49. (Withdrawn) The method of claim 48, wherein said cardiac specific mechanical contraction is selected from the group consisting of spontaneous

mechanical contraction, rhythmic mechanical contraction, synchronous mechanical contraction, and propagative mechanical contraction.

50. (Withdrawn) The method of claim 48, wherein said cardiac specific structure is selected from the group consisting of a sarcomere, a Z-band, a Z-body, an intercalated disc, a gap junction, a desmosome, a fibrillar bundle, a fibrillar bundle striation, and a myocytic syncytium.

51. (Withdrawn) The method of claim 48, wherein said cardiac specific RNA encodes a protein selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, .alpha.-actinin, cardiac troponin I, cardiac troponin T, GATA-4, Nkx2.5, MLC-2A, MLC-2V, atrial myosin light chain, ventricular myosin light chain, and connexin-43.

52. (Withdrawn) The method of claim 48, wherein said cardiac specific protein is selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, atrial natriuretic peptide, cardiac troponin I, desmin and connexin-43.

53. (Withdrawn) The method of claim 48, wherein said cardiac specific electrical activity is selected from the group consisting of spontaneous electrical activity, rhythmic electrical activity, synchronized electrical activity, and propagative electrical activity.

54. (Withdrawn) The method of claim 53, wherein said propagative electrical activity is characterized by slow conduction.

55. (Withdrawn) A method of characterizing a biological state or a biological process of cardiac cells or cardiac tissue, the method comprising: (a) partially

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dispersing a confluent cultured population of human stem cells, thereby generating a cell population including cell aggregates; (b) subjecting said cell aggregates to culturing conditions suitable for generating embryoid bodies; (c) subjecting said embryoid bodies to culturing conditions suitable for inducing cardiac lineage differentiation in at least a portion of the cells of said embryoid bodies thereby generating cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or tissue predominantly displaying at least one characteristic associated with a cardiac phenotype; and (d) obtaining data characterizing the biological state or the biological process in said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype.

56. (Withdrawn) The method of claim 55, wherein said culturing conditions suitable for inducing cardiac lineage differentiation include adherence of said embryoid bodies to a surface.

57. (Withdrawn) The method of claim 55, further comprising isolating said cell aggregates from said cell population prior to step (b).

58. (Withdrawn) The method of claim 55, further comprising isolating said embryoid bodies prior to step (c).

59. (Withdrawn) The method of claim 55, wherein said culturing conditions suitable for inducing cardiac lineage differentiation further include culture medium supplemented with serum.

60. (Withdrawn) The method of claim 55, further comprising screening and optionally isolating cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or tissue predominantly displaying at least one

characteristic associated with a cardiac phenotype, said screening is effected by at least one method selected from the group consisting of detection of mechanical contraction, detection of a cardiac specific structure, detection of a cardiac specific protein, detection of a cardiac specific RNA, detection of cardiac specific electrical activity, and detection of cardiac specific changes in the intracellular concentration of a physiological ion.

61. (Withdrawn) The method of claim 60, wherein said detection of cardiac specific electrical activity is effected using a microelectrode array.

62. (Withdrawn) The method of claim 61, wherein said multielectrode array comprises electrodes positioned 100 .mu.m or less apart.

63. (Withdrawn) The method of claim 61, wherein said multielectrode array comprises at least 60 electrodes.

64. (Withdrawn) The method of claim 61, wherein said multielectrode array is configured to obtain data character said cardiac specific electrical activity with a frequency greater than a range selected from 1-25 kHz.

65. (Withdrawn) The method of claim 60, further comprising screening and optionally isolating cells substantially displaying proliferation or tissue substantially displaying proliferation.

66. (Withdrawn) The method of claim 55, further comprising inducing the biological state or the biological process in said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype.

67. (Withdrawn) The method of claim 66, wherein said inducing the biological state or the biological process is effected by treating said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype with a treatment selected from the group consisting of a treatment with a drug, a treatment with a physiological ion, and an electrical treatment.

68. (Withdrawn) The method of claim 67, wherein said drug is selected from the group consisting of 1-heptanol, isoproterenol, carbamylcholine, forskolin, IBMX, atropine, tetrodotoxin, and diltiazem hydrochloride

69. (Withdrawn) The method of claim 67, wherein said physiological ion is selected from the group consisting of a potassium ion, a sodium ion, and a calcium ion.

70. (Withdrawn) The method of claim 55, further comprising co-culturing said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype with primary cardiac cells or primary cardiac tissue prior to step (d).

71. (Withdrawn) The method of claim 55, further comprising transplanting said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype into cardiac tissue of a recipient prior to step (d).

72. (Withdrawn) The method of claim 71, wherein said recipient is a swine.

73. (Withdrawn) The method of claim 55, wherein said human stem cells are embryonic stem cells

74. (Withdrawn) The method of claim 55, wherein said partially dispersing a confluent cultured population of human stem cells is effected via a non-trypsin based method.

75. (Withdrawn) The method of claim 55, wherein said partially dispersing a confluent cultured population of human stem cells is effected via treatment with collagenase.

76. (Withdrawn) The method of claim 55, wherein said culturing in step (b) is effected for a time period selected from the range of 1 to 20 days.

77. (Withdrawn) The method of claim 55, wherein said culturing conditions in step (b) include inhibiting adherence of said cell aggregates a surface.

78. (Withdrawn) The method of claim 55, wherein said culturing conditions in step (b) include culture medium supplemented with serum

79. (Withdrawn) The method of claim 55, wherein said culturing in step (c) is effected for at least as long as a time period selected from the group consisting of 1-60 days.

80. (Withdrawn) The method of claim 55, wherein said culturing in step (c) is effected in the presence of dimethyl sulfoxide.

81. (Withdrawn) The method of claim 56, wherein said culturing conditions include exposing said embryoid bodies to a surface coated with gelatin.

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82. (Withdrawn) The method of claim 55, wherein said at least one characteristic associated with a cardiac phenotype to selected from the group consisting of cardiac specific mechanical contraction, a cardiac specific structure, expression of a cardiac specific RNA, expression of a cardiac specific protein, cardiac specific changes in the intracellular concentration of a physiological ion, and cardiac specific electrical activity.

83. (Withdrawn) The method of claim 82, wherein said cardiac specific mechanical contraction is selected from the group consisting of spontaneous mechanical contraction, rhythmic mechanical contraction, synchronous mechanical contraction, and propagative mechanical contraction.

84. (Withdrawn) The method of claim 82, wherein said cardiac specific structure is selected from the group consisting of a sarcomere, a Z-band, a Z-body, an intercalated disc, a gap junction, a desmosome, a fibrillar bundle, a fibrillar bundle striation, and a myocytic syncytium.

85. (Withdrawn) The method of claim 82, wherein said cardiac specific RNA encodes a protein selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, .alpha.-actinin, cardiac troponin I, cardiac troponin T, GATA-4, Nkx2.5 MLC-2A, MLC-2V, atrial myosin light chain, ventricular myosin light chain, and connexin-43.

86. (Withdrawn) The method of claim 82, wherein said cardiac specific protein is selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, atrial natriuretic peptide, cardiac troponin I, desmin and connexin-43.

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87. (Withdrawn) The method of claim 82, wherein said cardiac specific electrical activity is selected from the group consisting of spontaneous electrical activity, rhythmic electrical activity, synchronized electrical activity, and propagative electrical activity.

88. (Withdrawn) The method of claim 87, wherein said propagative electrical activity is characterized by slow conduction.

89. (Withdrawn) The method of claim 55, wherein the biological state or the biological process is selected from the group consisting of cardiac specific mechanical contraction, a cardiac specific structure, expression of a cardiac specific RNA, expression of a cardiac specific protein, cardiac specific changes in the intracellular concentration of a physiological ion, cardiac specific electrical activity, and cardiomyogenesis.

90. (Withdrawn) The method of claim 89, wherein said cardiac specific mechanical contraction is selected from the group consisting of spontaneous mechanical contraction, rhythmic mechanical contraction, synchronous mechanical contraction, propagative mechanical contraction, and arrhythmic cardiac contraction.

91. (Withdrawn) The method of claim 89, wherein said cardiac specific structure is selected from the group consisting of a sarcomere, a Z-band, a Z-body, an intercalated disc, a gap junction, a desmosome, a fibrillar bundle, a fibrillar bundle striation, and a myocytic syncytium.

92. (Withdrawn) The method of claim 89, wherein said cardiac specific RNA encodes a protein selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, .alpha.-actinin, cardiac troponin I, cardiac

troponin T, GATA-4, Nkx2.5, MLC-2A, MLC-2V, atrial myosin light chain, ventricular myosin light chain, and connexin-43.

93. (Withdrawn) The method of claim 89, wherein said cardiac specific protein is selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, atrial natriuretic peptide, cardiac troponin I, desmin and connexin-43

94. (Withdrawn) The method of claim 89, wherein said cardiac specific electrical activity is selected from the group consisting of spontaneous electrical activity, rhythmic electrical activity, synchronized electrical activity, and propagative electrical activity.

95. (Withdrawn) The method of claim 94, wherein said propagative electrical activity is characterized by slow conduction.

96. (Withdrawn) The method of claim 55, wherein the biological state or the biological process is cardiac specific electrical activity and whereas said obtaining data characterizing the biological state or the biological process is effected using a multielectrode array.

97. (Withdrawn) The method of claim 96, wherein said multielectrode array comprises electrodes positioned 100 .mu.m or less apart.

98. (Withdrawn) The method of claim 96, wherein said multielectrode array comprises at least 60 electrodes.

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99. (Withdrawn) The method of claim 96, wherein said multielectrode array is configured to obtain data characterizing said cardiac specific electrical activity with a frequency greater than a range selected from 1-25 kHz.

100. (Withdrawn) A method of qualifying the effect of a treatment on a biological state or a biological process of cardiac cells or cardiac tissue, the method comprising: (a) partially dispersing a confluent cultured population of human stem cells, thereby generating a cell population including cell aggregates; (b) subjecting said cell aggregates to culturing conditions suitable for generating embryoid bodies; (c) subjecting said embryoid bodies to culturing conditions suitable for inducing cardiac lineage differentiation in at least a portion of the cells of said embryoid bodies thereby generating cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or tissue predominantly displaying at least one characteristic associated with a cardiac phenotype; (d) subjecting said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype to the treatment; and (e) monitoring the biological state or the biological process in said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype, thereby qualifying the effect of the treatment on the biological state or the biological process.

101. (Withdrawn) The method of claim 100, wherein said culturing conditions suitable for inducing cardiac lineage differentiation include adherence of said embryoid bodies to a surface.

102. (Withdrawn) The method of claim 100, wherein the treatment is effected by subjecting said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one

characteristic associated with a cardiac phenotype to an exposure to a compound or to an electrical treatment.

103. (Withdrawn) The method of claim 100, further comprising isolating said cell aggregates from said cell population prior to step (b).

104. (Withdrawn) The method of claim 100, further comprising isolating said embryoid bodies prior to step (c).

105. (Withdrawn) The method of claim 100, wherein said culturing conditions suitable for inducing cardiac lineage differentiation further include culture medium supplemented with serum.

106. (Withdrawn) The method of claim 100, further comprising screening and optionally isolating cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or tissue predominantly displaying at least one characteristic associated with a cardiac phenotype, said screening effected by at least one method selected fin the group consisting of detection of mechanical contraction, detection of a cardiac specific structure, detection of a cardiac specific protein, detection of a cardiac specific RNA, detection of cardiac specific electrical activity, and detection of cardiac specific changes in the intracellular concentration of a physiological ion.

107. (Withdrawn) The method of claim 106, further comprising screening and optionally isolating cells substantially displaying proliferation or tissue substantially displaying proliferation.

108. (Withdrawn) The method of claim 100, further comprising inducing the biological state or the biological process in said cells predominantly displaying at least

one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype.

109. (Withdrawn) The method of claim 108, wherein said Inducing the biological state or the biological process is effected by treating said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype with a treatment selected from the group consisting of a treatment with a drug, a treatment with a physiological ion, and an electrical treatment.

110. (Withdrawn) The method of claim 109, wherein said drug is selected from the group consisting of 1-heptanol, isoproterenol, carbamylcholine, forskolin, IBMX, atropine, tetrodotoxin, and diltiazem hydrochloride

111. (Withdrawn) The method of claim 109, wherein said physiological ion is selected from the group consisting of a potassium ion, a sodium ion, and a calcium ion.

112. (Withdrawn) The method of claim 100, further comprising co-culturing said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype with primary cardiac cells or primary cardiac tissue following step (c).

113. (Withdrawn) The method of claim 100, further comprising transplanting said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic

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associated with a cardiac phenotype into cardiac tissue of a recipient following step (c).

114. (Withdrawn) The method of claim 113, wherein said recipient is a swine.

115. (Withdrawn) The method of claim 100, wherein said human stem cells are embryonic stem cells.

116. (Withdrawn) The method of claim 100, wherein said partially dispersing a confluent cultured population of human stem cells is effected via a non-trypsin based method.

117. (Withdrawn) The method of claim 100, wherein said partially dispersing a confluent cultured population of human stem-cells is effected via treatment with collagenase.

118. (Withdrawn) The method of claim 100, wherein said culturing in step (b) is effected for a time period selected from the range of 1 to 20 days.

119. (Withdrawn) The method of claim 100, wherein said culturing conditions in step (b) include inhibiting adherence of said cell aggregates to a surface.

120. (Withdrawn) The method of claim 100, wherein said culturing conditions in step (b) include culture medium supplemented with serum.

121. (Withdrawn) The method of claim 100, wherein said culturing in step (c) is effected for at least as long as a time period selected from the range of 1-60 days.

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122. (Withdrawn) The method of claim 100, wherein said culturing in step (c) is effected in the presence of dimethyl sulfoxide.

123. (Withdrawn) The method of claim 101, wherein said culturing conditions include exposing said embryoid bodies to a surface coated with gelatin.

124. (Withdrawn) The method of claim 100, wherein said at least one characteristic associated with a cardiac phenotype is selected from the group consisting of cardiac specific mechanical contraction, a cardiac specific structure, expression of a cardiac specific RNA, expression of a cardiac specific protein, cardiac specific changes in the intracellular concentration of a physiological ion, and cardiac specific electrical activity.

125. (Withdrawn) The method of claim 124, wherein said cardiac specific mechanical contraction is selected from the group consisting of spontaneous mechanical contraction, rhythmic mechanical contraction, synchronous mechanical contraction, and propagative mechanical contraction.

126. (Withdrawn) The method of claim 124, wherein said cardiac specific structure is selected from the group consisting of a sarcomere, a Z-band, a Z-body, an intercalated disc, a gap junction, a desmosome, a fibrillar bundle, a fibrillar bundle striation, and a myocytic syncytium.

127. (Withdrawn) The method of claim 124, wherein said cardiac specific RNA encodes a protein selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, .alpha.-actinin, cardiac troponin I, cardiac troponin T, GATA-4, Nkx2.5, MLC-2A, MLC.2V, atrial myosin light chain, ventricular myosin light chains and connexin-43.

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128. (Withdrawn) The method of claim 124, wherein said cardiac specific protein is selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, atrial natriuretic peptide, cardiac troponin I, desmin and connexin-43.

129. (Withdrawn) The method of claim 124, wherein said cardiac specific electrical activity is selected from the group consisting of spontaneous electrical activity, rhythmic electrical activity, synchronized electrical activity, and propagative electrical activity.

130. (Withdrawn) The method of claim 129, wherein said propagative electrical activity is characterized by slow conduction.

131. (Withdrawn) The method of claim 100, wherein the biological state or the biological process is selected from the group consisting of cardiac specific mechanical contraction, a cardiac specific structure, expression of a cardiac specific RNA, expression of a cardiac specific protein, cardiac specific changes in the intracellular concentration of a physiological ion, cardiac specific electrical activity, and cardiomyogenesis.

132. (Withdrawn) The method of claim 131, wherein said cardiac specific mechanical contraction is selected from the group consisting of spontaneous mechanical contraction, rhythmic mechanical contraction, synchronous mechanical contraction, propagative mechanical contraction, and arrhythmic cardiac contraction.

133. (Withdrawn) The method of claim 131, wherein said cardiac specific structure is selected from the group consisting of a sarcomere, a Z-band, a Z-body, an intercalated disc, a gap junction, a desmosome, a fibrillar bundle, a fibrillar bundle striation, and a myocytic syncytium.

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134. (Withdrawn) The method of claim 131, wherein said cardiac specific RNA encodes a protein selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, .alpha.-actinin, cardiac troponin I, cardiac troponin T, GATA-4, Nkx2.5, MLC2A, MLC-2V, atrial myosin light chain, ventricular myosin light chain, and connexin-43.

135. (Withdrawn) The method of claim 131, wherein said cardiac specific protein is selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, atrial natriuretic peptide, cardiac troponin I, desmin and connexin-43.

136. (Withdrawn) The method of claim 131, wherein said cardiac specific electrical activity is selected from the group consisting of spontaneous electrical activity, rhythmic electrical activity, synchronized electrical activity, and propagative electrical activity.

137. (Withdrawn) The method of claim 136, wherein said propagative electrical activity is characterized by slow conduction.

138. (Withdrawn) The method of claim 100, wherein the biological state or the biological process is cardiac specific electrical activity and whereas said monitoring the biological state or the biological process is effected using a multielectrode array.

139. (Withdrawn) The method of claim 138, wherein said multielectrode array comprises electrodes positioned 100 .mu.m or less apart.

140. (Withdrawn) The method of claim 138, wherein said multielectrode array comprises at least 60 electrodes.

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141. (Withdrawn) The method of claim 138, wherein said multielectrode array measures electrical activity with a frequency of 10 kHz or higher.

142. (Withdrawn) A method of repairing cardiac tissue in a subject, the method comprising: (a) partially dispersing a confluent cultured population of human stem cells, thereby generating a cell population including cell aggregates; (b) subjecting said cell aggregates to culturing conditions suitable for generating embryoid bodies; (c) subjecting said embryoid bodies to culturing conditions suitable for inducing cardiac lineage differentiation in at least a portion of the cells of said embryoid bodies thereby generating cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or tissue predominantly displaying at least one characteristic associated with a cardiac phenotype; and (d) administering a therapeutically effective dose of said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, and/or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype to the heart of the subject, thereby repairing cardiac tissue in the subject.

143. (Withdrawn) The method of claim 142, wherein said culturing conditions suitable for inducing cardiac lineage differentiation include adherence of said embryoid bodies to a surface.

144. (Withdrawn) The method of claim 142, further comprising isolating said cell aggregates from said cell population prior to step (b).

145. (Withdrawn) The method of claim 142, further comprising isolating said embryoid bodies prior to step (c).

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146. (Withdrawn) The method of claim 142, wherein said culturing conditions suitable for inducing cardiac lineage differentiation flier include culture medium supplemented with serum.

147. (Withdrawn) The method of claim 142, further comprising screening and optionally isolating cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or tissue predominantly displaying at least one characteristic associated with a cardiac phenotype, said screening effected by at least one method selected from the group consisting of detection of mechanical contraction, detection of a cardiac specific structure, detection of a cardiac specific protein, detection of a cardiac specific RNA, detection of cardiac specific electrical activity, and detection of cardiac specific changes in the concentration of intracellular calcium ion.

148. (Withdrawn) The method of claim 6, wherein said detection of cardiac specific electrical activity is effected using a microelectrode array.

149. (Withdrawn) The method of claim 148, wherein said multielectrode array comprises electrodes positioned 100 .mu.m or less apart.

150. (Withdrawn) The method of claim 148, wherein said multielectrode array comprises at least 60 electrodes.

151. (Withdrawn) The method of claim 148, wherein said multielectrode array is configured to obtain data characterizing said cardiac specific electrical activity with a frequency greater than a range selected from 1-25 kHz.

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152. (Withdrawn) The method of claim 147, fixer comprising screening and optionally isolating cells substantially displaying proliferation or tissue substantially displaying proliferation.

153. (Withdrawn) The method of claim 142, fix comprising treating the subject with an immunosuppressive regimen, thereby promoting engraftment of said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype in the subject.

154. (Withdrawn) The method of claim 142, wherein said administering is effected by injection of said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype into the heart of the subject.

155. (Withdrawn) The method of claim 142, further comprising inactivating or removing pathogenic cardiac cells or cardiac tissue in the subject

156. (Withdrawn) The method of claim 142, wherein said human stem cells are embryonic stem cells.

157. (Withdrawn) The method of claim 142, wherein said human stem cells are syngeneic with the subject.

158. (Withdrawn) The method of claim 142, wherein said partially dispersing a confluent cultured population of human stem cells is effected via a non-trypsin based method.

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159. (Withdrawn) The method of claim 142, wherein said partially dispersing a confluent cultured population of human stem cells is effected via treatment with collagenase.

160. (Withdrawn) The method of claim 142, wherein said culturing in step (b) is effected for a time period selected from the range of 1 to 20 days.

161. (Withdrawn) The method of claim 142, wherein said culturing conditions in step (b) include inhibiting adherence of said cell aggregates to a surface.

162. (Withdrawn) The method of claim 142, wherein said culturing conditions in step (b) include culture medium supplemented with serum.

163. (Withdrawn) The method of claim 142, wherein said culturing in step (c) is effected for at least as long as a time period selected from the range of 1-60 days.

164. (Withdrawn) The method of claim 142, wherein said culturing in step (e) is effected in the presence of dimethyl sulfoxide.

165. (Withdrawn) The method of claim 142, wherein said culturing conditions in step (c) include exposing said embryoid bodies to a surface coated with gelatin.

166. (Withdrawn) The method of claim 142, wherein said at least one characteristic associated with a cardiac phenotype is selected from the group consisting of cardiac specific mechanical contraction, a cardiac specific structure, expression of a cardiac specific RNA, expression of a cardiac specific protein, cardiac specific changes in the intracellular concentration of a physiological ion, and cardiac specific electrical activity.

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167. (Withdrawn) The method of claim 166, wherein said cardiac specific mechanical contraction is selected from the group consisting of spontaneous mechanical contraction, rhythmic mechanical contraction, synchronous mechanical contraction, and propagative mechanical contraction.

168. (Withdrawn) The method of claim 166, wherein said cardiac specific structure is selected from the group consisting of a sarcomere, a Z-band, a Z-body, an intercalated disc, a gap junction, a desmosome, a fibrillar bundle, a fibrillar bundle striation, and a myocytic syncytium.

169. (Withdrawn) The method of claim 166, wherein said cardiac specific RNA encodes a protein selected from the group consisting of cardiac .alpha.myosin heavy chain, cardiac .beta.-myosin heavy chain, .alpha.-actinin, cardiac troponin I, cardiac troponin T, GATA-4, Nkx2.5, MLC-2A, MLC-2V, atrial myosin light chain ventricular myosin light chain, and connexin-43.

170. (Withdrawn) The method of claim 166, wherein said cardiac specific protein is selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, atrial natriuretic peptide, cardiac troponin I, desmin and connexin-43.

171. (Withdrawn) The method of claim 166, wherein said cardiac specific electrical activity is selected from the group consisting of spontaneous electrical activity, rhythmic electrical activity, synchronized electrical activity, and propagative electrical activity.

172. (Withdrawn) The method of claim 142, wherein the subject is a human or a nonhuman mammal.

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173. (Withdrawn) The method of claim 142, wherein the subject has a cardiac disorder characterized by cardiac arrhythmia, and whereas said administering is effected by intra-myocardial injection of said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype, thereby treating said disorder characterized by cardiac arrhythmia.

174. (Withdrawn) The method of claim 142, wherein the subject has a cardiac disorder characterized by abnormal generation of the electrical impulse or impaired conduction and whereas said administering is effected by intra-myocardial injection of said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype, thereby treating said disorder characterized by impaired cardiac conducting tissue.

175. (Withdrawn) The method of claim 142, wherein the subject has a cardiac disorder characterized by myocardial ischemia, and whereas said administering is effected by intra-myocardial injection of said cells predominantly displaying at least one characteristic associated with a cardiac phenotype, or said tissue predominantly displaying at least one characteristic associated with a cardiac phenotype, thereby treating said disorder characterized by myocardial ischemia.

176. (Currently Amended) An in-vitro culture comprising a plurality of embryoid bodies which comprise ~~of isolated~~ human cells said plurality of embryoid bodies exhibiting ~~which will display substantial proliferation for at least as long as a time period selected from the range of 1-35 days, and which will predominantly display~~ at least one characteristic associated with a cardiac phenotype ~~for at least as long as a time period selected from the range of 1-60 days~~.

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177. (Original) The in-vitro culture of claim 176, wherein said at least one characteristic associated with a cardiac phenotype is selected from the group consisting of mechanical contraction, a cardiac specific structure, a cardiac specific protein, a cardiac specific RNA, cardiac specific electrical activity, cardiac specific changes in the intracellular concentration of a physiological ion, and cardiomyogenesis.

178. (Currently amended) The in-vitro culture of claim 177, wherein said ~~isolated human cells~~ plurality of embryoid bodies are cultured in contact with a multielectrode array configured for monitoring said cardiac specific electrical activity.

179. (Currently Amended) The ~~method~~ in vitro culture of claim 178, wherein said multielectrode array comprises electrodes positioned 100 .mu.m or less apart.

180. (Currently Amended) The ~~method~~ in vitro culture of claim 178, wherein said multielectrode array comprises at least 60 electrodes.

181. (Currently Amended) The ~~method~~ in vitro culture of claim 178, wherein said multielectrode array is configured to obtain data characterizing said cardiac specific electrical activity with a frequency greater than a range selected from 1-25 kHz.

182. (Withdrawn) The in-vitro culture of claim 177, wherein said cardiac specific mechanical contraction is selected from the group consisting of spontaneous mechanical contraction, rhythmic mechanical contraction, synchronous mechanical contraction, and propagative mechanical contraction.

183. (Withdrawn) The in-vitro culture of claim 177, wherein said cardiac specific structure is selected from the group consisting of a sarcomere, a Z-band, a Z-

body, an intercalated disc, a gap junction, a desmosome, a fibrillar bundle, a fibrillar bundle striation, and a myocytic syncytium.

184. (Withdrawn) The in-vitro culture of claim 177, wherein said cardiac specific RNA encodes a protein selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, .alpha.-actinin, cardiac troponin I, cardiac troponin T, GATA-4, Nkx2.5, MLC-2A, MLC-2V, atrial myosin light chain, ventricular myosin light chain, and connexin-43.

185. (Withdrawn) The in-vitro culture of claim 177, wherein said cardiac specific protein is selected from the group consisting of cardiac .alpha.-myosin heavy chain, cardiac .beta.-myosin heavy chain, atrial natriuretic peptide, cardiac troponin I, desmin and connexin-43.

186. (Original) The in-vitro culture of claim 177, wherein said cardiac specific electrical activity is selected from the group consisting of spontaneous electrical activity, rhythmic electrical activity, synchronized electrical activity, and propagative electrical activity.

187-195. (Canceled)

196. (New) The in-vitro culture of claim 176, wherein at least 20-60 % of said human cells exhibit proliferation

197. (New) The in-vitro culture of claim 177, wherein said proliferation is for at least as long as a time period selected from the range of 1-35 days

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198. (New) The in-vitro culture of claim 176, wherein said displaying of said at least one characteristic associated with a cardiac phenotype is for at least as long as a time period selected from the range of 1-60 days.

199. (New) The in vitro culture of claim 176, wherein said plurality of embryoid bodies comprise sections of embryoid bodies.